

## Identification of *Brucella abortus* Antibodies in Cattle Serum by Single Radial Diffusion

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Single radial diffusion combined with the Rose Bengal test permitted rapid identification of all of the *Brucella abortus*-infected cattle in a study group of 689 animals.

The single radial diffusion (SRD) technique of Mancini et al. (8) is simple and rapidly performed and its sensitivity is remarkable (3-5). This technique also permits each distinct immune system to be identified. These factors prompted an evaluation of the usefulness of the SRD technique for diagnosis of *Brucella* infection in cattle.

The brucellae (*Brucella abortus* Weybridge 99, virulent and of smooth colonial morphology) grown on agar were suspended in saline (approximately  $2.5 \times 10^{12}$  bacteria per ml) and sonicated for 15 min at 4 C with an MSE 60 W ultrasonic disintegrator. The suspension was centrifuged immediately at 4 C for 30 min at  $8 \times 10^4 \times g$ , and the supernatant fluid constituted the antigen preparation (AG-I). The agglutinogens A and M, referred to in this article as AG-II antigen, were extracted using hot saline (10); *B. melitensis* 16 M was used instead of *B. abortus* because of better diffusion in agar gels (2). The reversed version of the conventional single radial immunodiffusion developed by Vaerman et al. (11) was adopted, and the agar plates were prepared as described by Mancini et al. (8). Each plate (12 by 9 cm) was filled with 7 ml of the following mixture: 3% (wt/vol) agarose (Behringwerke-AG, Marburg-Lohn) in glycine buffer (pH 7.8), 3.5 ml; AG-I, 0.25 ml; AG-II, 0.25 ml; glycine buffer (pH 7.8), 3 ml. The wells (2.5 mm in diameter) were filled with 4  $\mu$ l of individual cattle serum samples. The plates were incubated in a humidified chamber at room temperature for 2 days. The complement fixation (CF) test was carried out as described by Alton and Jones (1) using 0.25 ml of serum to be tested diluted in 2-fold steps to 1:32 with barbital buffer prepared according to the method of Kabat and Mayer (7). The Rose Bengal test (RB) was carried out by mixing on a dark glass plate 1 drop (approximately 0.05 ml)

of the serum to be tested and 1 drop of antigen (obtained from the Central Veterinary Laboratory, Weybridge, Surrey).

Single serum samples collected from 689 animals were examined by SRD, CF, and the RB test. Figure 1 shows the reaction pattern in the SRD system of a strongly positive serum displaying as many as seven to eight different rings. On the other hand, weakly positive sera gave a very faint antigen-antibody reaction. In these cases, a 4 $\times$  magnifying hand lens was found helpful for direct reading.

Table 1 summarizes the results obtained with the three methods. All serum samples positive by SRD were also positive in at least one of the two other methods. Clearly, the specificity of SRD is the same as that of CF and RB, which are accepted as reliable methods. Concerning the sensitivity of the three methods, the SRD test was the least sensitive, as it failed to detect 35 (12%) of 284 positive samples. From the results of Table 1 it is also clear that none of the three methods is 100% reliable in diagnosing *Brucella* infection. Hunter and Allen (6) reached the same conclusion after comparing two milk tests (milk ring and CF tests on the whole milk) and three blood tests (serum agglutination, CF, and RB tests).

A combination of the RB test and either of the other two methods identified all of the reactive animals in the study (Table 1). This observation points out the usefulness of a dual test system, already proposed by Miller (9). SRD might properly be used in place of the complex and time-consuming CF test as a rapid test in a dual system with the RB test for an accurate and quick identification of all reactive animals. The SRD test might also be used in special cases such as with anticomplementary sera, or when it is considered necessary to identify and quantitate the broad spectrum of antibodies

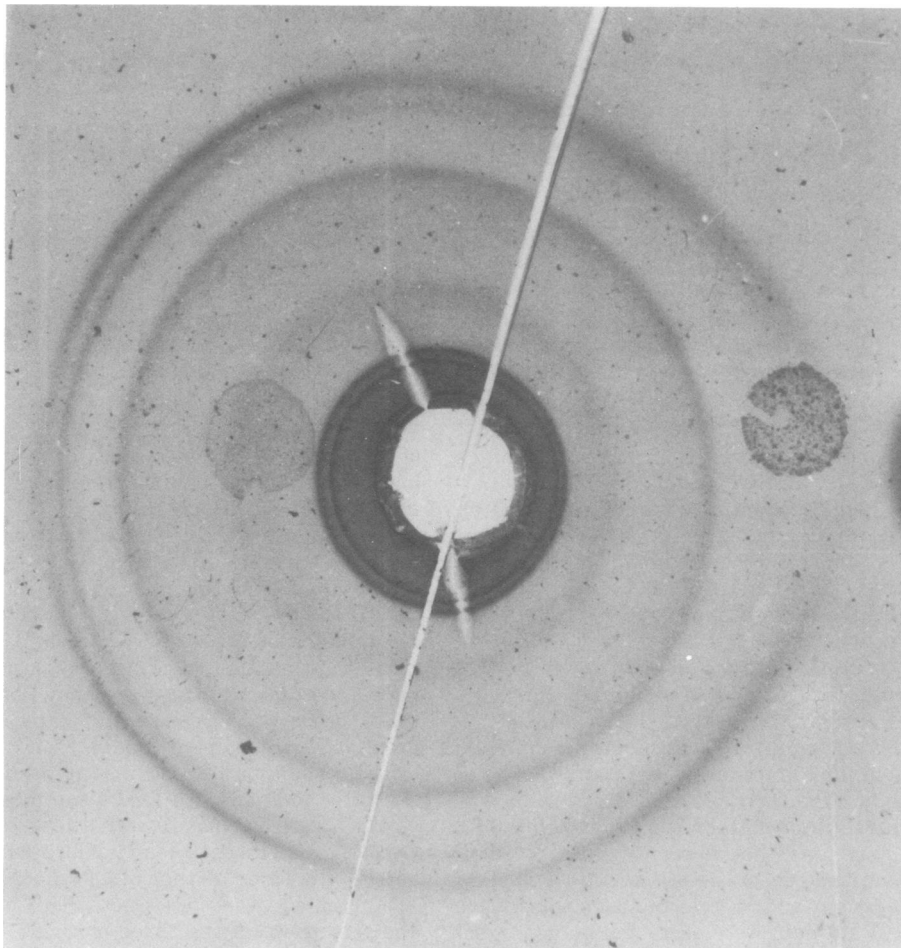


FIG. 1. SRD pattern produced with sera from an infected animal.

TABLE 1. Results obtained with the RB, CF, and SRD tests

RB	CF	SRD	No. of animals
+	+	+	237
-	-	-	405
+	+	-	25
+	-	-	10
-	+	+	5
+	-	+	7

formed by infected animals against the antigenic mosaic of *B. abortus*. Finally, this technique makes it theoretically possible to distinguish between antibodies resulting from infection with smooth-type *Brucella* and those resulting from vaccination, where a rough-type *Brucella* vaccine is used.

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